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Short communication

Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi

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Abstract

Five essential oils (EO) extracted from *Cymbopogon citratus*, *Monodora myristica*, *Ocimum gratissimum*, *Thymus vulgaris* and *Zingiber officinale* were investigated for their inhibitory effect against three food spoilage and mycotoxin producing fungi, *Fusarium moniliforme*, *Aspergillus flavus* and *Aspergillus fumigatus*. Five strains of each fungus were tested. The agar dilution technique was used to determine the inhibitory effect of each EO on the radial growth of the fungus, and a dose response was recorded. The EO from *O. gratissimum*, *T. vulgaris* and *C. citratus* were the most effective and prevented conidial germination and the growth of all three fungi on corn meal agar at 800, 1000 and 1200 ppm, respectively. Moderate activity was observed for the EO from *Z. officinale* between 800 and 2500 ppm, while the EO from *M. myristica* was less inhibitory. These effects against food spoilage and mycotoxin producing fungi indicated the possible ability of each essential oil as a food preservative. A comparative test on the preservative ability of the EO from *O. gratissimum* and potassium sorbate against *A. flavus* at pH 3.0 and 4.5 showed that the EO remained stable at both pH, whereas the efficacy of potassium sorbate was reduced at higher pH. We concluded that the EO from *O. gratissimum* is a potential food preservative with a pH dependent superiority against potassium sorbate, and these are novel scientific information.

Keywords: Essential oils; Food preservatives; Fungi; Growth inhibition; Radial growth

1. Introduction

Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries. *Penicillium*, *Aspergillus* and *Fusarium* are the most important fungi causing spoilage of African foodstuffs (Nickelsen and Jakobsen, 1997). Fungi are also responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses (Farag et al., 1989; Nielsen and Rios, 2000). Aflatoxin B_1 and B_2 and fumitoxins produced by *Aspergillus flavus* and *A. fumigatus* are some examples of mycotoxins produced by such fungi (Singh et al., 1991). A number of important mycotoxins have been isolated from *Fusarium moniliforme* including moniliformin, fuminosins and zearalenone (Thrane, 1988; Ngoko, 1999). Adequate control measures to prevent spoilage of grains and foodstuffs are

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essential to avoid contamination and minimise public health hazards.

Pauli and Knobloch (1987) and Mishra and Dubey (1994) demonstrated the antibacterial and antifungal properties of some essential oils including *Thymus vulgaris* and *Cymbopogon citratus*, commonly used in Africa to season foods, against food-borne micro-organisms. The food preservative ability of the EO from mustard and *Capsicum annum* were demonstrated by Nielsen and Rios (2000) and Dorantes et al. (2000).

This study was undertaken to investigate the antifungal activity and the food preservative ability of essential oils extracted from selected plants of the rich flora of Cameroon against spoilage and mycotoxin producing fungi (*F. moniliforme*, *A. flavus* and *A. fumigatus*).

2. Materials and methods

2.1. Fungal strains and cultures

The cultures used in this study were obtained from the Institute of Biotechnology, Danish Technical University (Lyngby), Denmark. These were five strains, each of *A. flavus*, IBT 3660, IBT 15606, IBT 15714, IBT 18438 and IBT 19412, *A. fumigatus*, IBT 16901, IBT 17328, IBT 20466, IBT 20886 and IBT 21712 and *F. moniliforme*, IBT 9490, IBT 9494, IBT 9495, IBT 9498 and IBT 9504. The cultures were preserved on silica gel beads and transferred to corn meal agar (CMA) plates for recovery before they were tested.

2.2. Spices and essential oil extraction

The essential oils (EO) tested were extracted by the hydrodistillation method using Clevenger's apparatus (Lamaty et al., 1987). They were from *C. citratus* (fresh leaves), *Monodora myristica* (dried seeds), *Ocimum gratissimum* (fresh leaves), *T. vulgaris* (fresh whole plant) and *Zingiber officinale* (fresh rhizomes). The recovered oils were dried over anhydrous sodium sulphate and stored in darkness at 4 °C. The yield of the essential oils as percent of plant material weight were as follows: 0.57%, 6.2%, 0.42%, 0.36% and 0.50% for the EO from *C. citratus*, *M. Myristica*, *O. gratissimum*, *T. vulgaris* and *Z. officinale,* respectively. The plants are grown for commercial use in the humid forest and highland zones in Cameroon and are used as food flavours.

2.3. Chemical food preservative

Potassium sorbate was obtained from Cheminova, Denmark. A stock solution (2%) was prepared in distilled water.

2.4. Preparation of the conidial suspension

Conidia were harvested from 7-day-old cultures by pouring a sterile 0.01% aqueous solution of Tween 80 onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia in the suspension was adjusted to approximately 10^6 conidia ml^{-1} using a Bürker-Türk counting chamber (Heamocytometer).

2.5. Study of the antifungal properties of the five essential oils

The essential oils were assayed for their antifungal activities on the radial growth of mycelia, using an agar dilution method (Benjilali et al., 1986). The EOs were emulsified at the ratio of 1:9 (v/v) in 0.1% water–agar and used as stock solution. The EO concentrations ranging from 200 to 2500 ppm were prepared by adding an appropriate volume of the emulsion to the corresponding volume of CMA maintained in molten state (Table 1). The mixture was poured in Petri dishes and after solidification, 5 μl of the conidial suspension (10^6 conidia ml^{-1}) were inoculated at the centre of each CMA plates. For each concentration, 12 agar plates were inoculated with the fungus in four replicates of three plates each. After inoculation, all the CMA plates were incubated at 23 °C under a diurnal cycle of 12 h Near UV light and 12 hours darkness. The incubation periods were 7 and 8–14 days. The experiment was repeated twice. The radial growth of the mycelium was recorded at intervals until the fungal growth reached the edges of the control plates. The effect of the EO was evaluated by the percentage mycelial growth inhibition, using the formula of Pandey et al. (1982). The minimum inhibitory concentration (MIC) was assessed as the lowest concentration of the EO required for complete

Table 1
Effect of essential oils on the growth of the three fungi tested

Mean diameter ± SD of mycelial growth in mm				
Essential oils		Fungal species		
Plant name	Concentration (ppm)	*Fusarium moniliforme*	*Aspergillus flavus*	*Aspergillus fumigatus*
Ocimum gratissimum	200	$11.9^{d} \pm 0.8$	$28.7^{j} \pm 5.9$	$22.2^{h} \pm 5.0$
	400	$2.0^{b} \pm 2.8$	–	–
	500	$0.0^{a} \pm 0.0$	$5.7^{c} \pm 0.7$	$2.0^{b} \pm 2.7$
	600	–	$3.0^{b} \pm 2.7$	$0.0^{a} \pm 0.0$
	800	–	$0.0^{a} \pm 0.0$	–
Thymus vulgaris	200	$16.6^{e} \pm 1.0$	–	$25.6^{i} \pm 5.1$
	500	$0.0^{a} \pm 0.0$	$6.3^{c} \pm 1.2$	$2.2^{b} \pm 3.0$
	600	–	–	$0.0^{a} \pm 0.0$
	800	–	$2.0^{b} \pm 2.7$	–
	1000	–	$0.0^{a} \pm 0.0$	–
Cymbopogon citratus	300	$0.0^{a} \pm 0.0$	–	$27.0^{i} \pm 15.5$
	500	$0.0^{a} \pm 0.0$	$43.3^{n} \pm 4.8$	$7.1^{c} \pm 11.8$
	800	–	$19.1^{f} \pm 9.9$	$3.5^{b} \pm 7.9$
	1200	–	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$
Zingiber officinale	500	$26.5^{i} \pm 6.7$	$50.4^{p} \pm 5.2$	$36.6^{m} \pm 14.1$
	800	$0.0^{a} \pm 0.0$	–	–
	1000	–	$30.4^{k} \pm 6.9$	$10.7^{d} \pm 16.1$
	2000	–	15.3e ± 13.4	$7.1^{c} \pm 15.8$
Monodora myristica	500	$66.5^{r} \pm 1.9$	$74.2^{s} \pm 1.9$	$75.8^{s} \pm 3.5$
Control	0	85.0 ± 0.0	84.0 ± 2.2	84.0 ± 2.2

Values in the same column followed by a different letter are significantly different ($P = 0.01$).
Data are Means ± SD of five replicates. Each replicate is average of four recordings from two separate experiments.
–: Not tested.

inhibition of the visible growth of the fungus. After asssessment, the fungicidal effect of the EO was determined by transferring agar plugs on which the inoculum was deposited from the CMA with EO, to CMA without EO and then incubated as previously described.

2.6. Study of the food preservative ability of the essential oil from O. gratissimum

A. flavus IBT 18438 was used as test fungus for the evaluation of the preservative ability of the EO from *O. gratissimum* on the colony counts in comparison to that of potassium sorbate at pH 3.0 and 4.5. The EOs were tested at the concentrations of 200 and 500 ppm. The potassium sorbate was tested at 100 and 200 ppm according to Lueck (1980). The conidial suspension of the fungus was prepared as previously described. From the suspension, four ten-fold serial dilutions were made and 50 μl aliquots from each dilution were plated in triplicates on CMA containing EO and controls without antifungal agents. The experiment was repeated twice, and plate counts were determined as colony forming units (CFU) per ml suspension of *A. flavus* after incubation at 25 ± 2 °C for 72 h in darkness. The fungicidal activity expressed as a number of decimal reduction of colony forming units per ml (NDR CFU) was calculated by the following formula:

$$\text{NDR CFU} = \log(N^{+}/N^{0})$$

N^{+} = CFU of *A. flavus* in treatment sets; N^{0} = CFU of *A. flavus* in control sets.

2.7. Statistical analysis

Significant differences between treatments and strains sensitivity were analysed using the M-STAT programme at 99% level of confidence. Mean separation test was done using the Least Significance Difference (LSD) (Nissen, 1990).

3. Results and discussion

Antifungal properties of the EO from the five plant species were recorded as inhibition of fungal mycelial growth and conidial germination (Table 1).

The EO from *O. gratissimum* at 200 ppm significantly reduced ($P=0.01$) the mycelial growth of *F. moniliforme*, *A. flavus* and *A. fumigatus* by 86%, 66% and 62%, respectively. Total inhibition of conidial germination of these fungi was observed at 500, 600 and 800 ppm, respectively (Table 1).

The EO from *T. vulgaris* at 200 ppm significantly ($P=0.01$) reduced the radial growth of *F. moniliforme* and *A. fumigatus* by 81% and 70%, respectively. At a concentration of 300 ppm, the mycelial growth of *A. flavus* was reduced by 81%. Total inhibition of conidial germination of *F. moniliforme*, *A. fumigatus* and *A. flavus* was 500, 600 and 1000 ppm, respectively (Table 1).

At 200 ppm, the EO from *C. citratus* reduced the mycelial growth of *F. moniliforme* by 64%. At 500 ppm, the radial growth of *A. flavus* and *A. fumigatus* was reduced by 48% and 77%, respectively. Total inhibition was obtained at 300 ppm for *F. moniliforme* and 1200 ppm for *A. flavus* and *A. fumigatus* (Table 1).

The EO from *Z. officinale* at 500 ppm significantly reduced the growth of *F. moniliforme*, *A. fumigatus* and *A. flavus* by 69%, 56% and 40%. Growth of *F. moniliforme* was totally inhibited by 800 ppm EO (Table 1); 2000 ppm was required to inhibit four of five strains of *A. fumigatus*, and only two of five strains of *A. flavus* were inhibited by EO concentration at 2500 ppm. At this concentration, growth reductions of 92%, 87% and 75%, were noted in the less sensitive strains (IBT 15714, IBT 18438 and IBT 19412), respectively.

About 500 ppm of EO from *M. myristica* reduced the growth of *F. moniliforme* by 22% (Table 1), however, radial growth of *A. fumigatus* and *A. flavus* was reduced 9.8% and 12%, but not statistically significant.

The EO from *O. gratissimum*, tested at concentrations of 200 and 500 ppm and at pH 3.0 and 4.5, totally inhibited the growth of *A. flavus* IBT 18438, while control plates showed growth of 10^6 CFU. Consequently, the NDR of CFU of the EO was more than six. This activity of EO from *O. gratissimum* was independent of pH, unlike that of the potassium sorbate (Table 2). At pH 4.5, 200 ppm of EO from *O. gratissimum* was over six fold more active (NDR CFU>6) than potassium sorbate (NDR CFU = 0.73), whereas at pH 3.0, both compounds expressed similar activity (NDR CFU>6) (Table 2).

This study reports on the antifungal properties of the essential oils from five plants tested against three food spoilage and mycotoxin producing fungi. Higher antifungal activity was found in the EO extracts from *C. citratus*, *O. gratissimum* and *T. vulgaris*. These EO may be potential antimicrobial compounds for use as food preservatives.

The EO from *O. gratissimum*, *T. vulgaris* and *C. citratus* significantly reduced ($P=0.01$) the growth of the five strains of *F. moniliforme*, *A. flavus* and *A. fumigatus* in a dosage response manner. The EO from *O. gratissimum* showed highest antifungal activity followed by *T. vulgaris* and *C. citratus*. The superiority of the EO from *O. gratissimum* against the three fungi demonstrated in this work is in agreement with the findings of Mishra et al. (1989) and Tagne et al. (2000), who worked withstrains of *A. flavus* and *F. moniliforme*, respectively. The first authors recorded a MIC of 2000 ppm against *A. flavus* using *O. gratissimum* EO from India. In the present study, a MIC of 800 ppm had been found with EO from the same plant species collected in Cameroon. This variability of the efficiency could be attributed to the difference in the EO chemical composition, which varies with the geographical location, time of harvest and the plant part collected (Ntezurubanza et al., 1987; Menut and Valet, 1985).

Antifungal activity of EO from *C. citratus* has previously been reported by Mishra and Dubey (1994) and Adegoke and Odesola (1996) and of EO from *O. gratissimum* and *T. vulgaris* against *A. flavus* by Pauli and Knobloch (1987) and Amvam Zollo et al. (1998).

Moderate activity between 800 and 2500 ppm was observed for the EO from *Z. officinale* while the EO

Table 2
Number of decimal reduction (NDR)[a] of CFU of *A. flavus* IBT 18438 by the EO from *O. gratissimum* and potassium sorbate

pH	K sorbate (ppm)		*O. gratissimum* (ppm)	
	100	200	200	500
3.0	0.95	>6	>6	>6
4.5	0.23	0.73	>6	>6

[a] Data are average of three replicates from two separate experiments.

from *M. myristica* was less inhibitory. Similar antifungal activity of EO from *Z. officinale* and *M. myristica* was reported by Hitokoto et al. (1980) and Awuah (1989).

The five EOs against the fungi tested expressed different levels of antifungal activities. This may be due to the differences in the content of known antimicrobial compounds in each EO as earlier determined by Lamaty et al. (1987) and Amvam Zollo et al. (1998). Farag et al. (1989) and Tassou et al. (2000) also discussed this correlation of the antifungal activity with the content of antimicrobial compounds of the EO. Studying the antifungal effect of five different EOs including thyme oil against *A. parasiticus*, Farag et al. (1989) concluded that the inhibitory effect of the oils was mainly due to the most abundant components. Menut and Valet (1985) and Amvam Zollo et al. (1998) reported a higher content of thymol in the EO from *O. gratissimum* (46.5%) compared to the EO from *T. vulgaris* (27.5%). According to Farag et al. (1989) this compound is highly inhibitory to microorganisms. This may explain the general superiority of the EO from *O. gratissimum* in the present work.

The comparative test of the EO from *O. gratissimum* with potassium sorbate against *A. flavus* showed that the EO remained effective at pH 3.0 and 4.5, whereas potassium sorbate was most effective at pH 3.0 and lost effectiveness with increasing pH from 3.0 to 4.5.

The present study demonstrated the potential food preservative ability of the EO from *O. gratissimum*, *T. vulgaris* and *C. citratus* against, *A. flavus*, *A. fumigatus* and *F. moniliforme*. These aromatic plants commonly used as spices or in beverage formulations are feasible as they are considered safe. However, further studies on their stability as food preservatives are needed here, including possibly their toxicity if used in the concentrations studied here.

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